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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JMH/7216-WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/02350	International filing date *(day/month/year)* 30.05.2003	Priority date *(day/month/year)* 31.05.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/00		
Applicant UNIVERSITY OF BRISTOL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.12.2003	26.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Stachowiak, O Telephone No. +49 89 2399-7219

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/02350

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-19 as originally filed

Claims, Numbers

1-15 filed with the demand

Drawings, Sheets

1/8-8/8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 14-15

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 14-15 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of report with regard to novelty, inventive step and industrial applicability

3.1 No examination report is provided with respect to claims **14-15** because these claims are so unclear that no meaningful opinion can be issued (Art 6 PCT; **cf. remarks provided infra in second written opinion**).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1 Reference is made to the following documents:

D1: OSBORNE, M. A. et al., XP002033515
D2: US 5,637,463 (DALTON et al.)
D3: VOLPERS C. et al., XP001154168
D4: CLARK D. D. et al., XP001154165
D5: KOCHAN J. P. et al., XP009016081
D6: SEREBRIISKII I. G. et al., XP009016134
D7: FULLER K. J. et al., XP001154167

5.2 The present set of claims fullfils the criteria of Art 34(2)(b) PCT.

NOVELTY:

5.3 With respect to claims 1-10:
None of the cited prior art documents discloses a method comprising a tribrid (trihybrid) system in which the prey comprises an antibody. Thus, claims 1-10 are novel.

5.4 The same applies with respect to the cells claimed in claims 11-13 which are also deemed novel.

INVENTIVE STEP:

5.5 With respect to claims 1-10:
Document D1 was chosen as closest prior art because it serves the same general purpose as the method of claim 1 and shares most of the features therewith. D1 discloses a screening method for regulatory enzymes comprising the construction

of a trihybrid (tribrid) cell comprising genes encoding an expression library of putative enzymes, a bait protein or polypeptide fused to a DNA binding domain, and a prey protein attached to an active domain recognising a post-translationally modified protein, being able to detect post-tranlationally modified bait proteins by induction of expression of a reporter gene (D1, p. 1475, col. 1, 2nd para. - p. 1478, col. 1, last para.; Fig. 1).

5.6 The difference between claim 1 and D1 is that the method of claim 1 as the **'prey'** employs a fusion protein between an antibody which recognizes a post-translationally modified protein, and a protein activation domain. The **technical effect** generated by that difference is that the method of claim 1 is not restricted to the detection of protein/protein interactions based on tyrosine phoyphorylation, but can be applied to more kinds of protein/protein interactions, and, furthermore, provides a fuctional screening system for regulatory enzymes of a protein of interest (cf. application, p. 2, 1st para. - p. 3, penultimate para.).

5.7 There is no hint in the cited prior art documents to employ a fusion between an antibody specific for a post-translationally modified protein fused to a protein activation domain as a 'prey' in a tribrid cell system. In view of the advantages specified in the description of the present application, the method of claim 1 is considered to involve an inventive step. The same applies to dependent claims 2-10 which relate to preferred embodiments of the method of claim 1.

5.8 With respect to claims 11-13:
Claims 11-13 are directed to a tribrid (tri-hybrid) cells reflecting the novel and inventive features of the respective methods. Thus, claims 11-13 are considered inventive for the reasons set out with respect to claims 1-10, supra.

5.9 With respect to claims 14-15:
Claims 14-15 are entirely unclear as they do not embrace any technical features. Thus, no examination report with respect to these claims is provided (cf. section 3.1, supra).

REPLACED BY ART 34 AMDT

CLAIMS

1. A screening method for regulatory enzymes, the method comprising the construction of a tribrid cell containing genes encoding an expression library of putative enzymes, a bait protein or polypeptide fused to a known DNA binding domain and a prey protein which recognises a protein or polypeptide which has been post translationally modified, the prey protein being attached to a known protein active domain, whereby, in use, binding or recognition of the bait protein or polypeptide by the prey protein or polypeptide upon post-translational modification by an enzyme contained in the expression library, causes transcription of a reporter gene or genes which allow recognition of the enzyme activity.

2. A method according to claim 1, in which the cell is a eukaryote.

3. A method according to claim 1 or claim 2, in which the cell is a yeast cell.

4. A method according to any one of claims 1 to 3, in which the enzyme is involved in the post-translational modification of nascent proteins or polypeptides.

5. A method according to claim 4, in which the enzyme is involved in the regulation of phosphorylation, glycosylation, sulphonation, acetylation, side chain modification, nitrosylation, ubiquination, myristoylation or palmitoylation.

6. A method according to claim 4 or claim 5, in which the enzyme is a kinase or a phosphatase.

7. A method according to any preceding claim, in which the bait protein is an oncoprotein, a kinase, a phosphatase, a receptor protein, an adapter protein or a scaffolding protein.

8. A method according to any preceding claim, in which the prey protein is conformationally constrained within the cell.

9. A method according to claim 8, in which the prey protein is conformationally constrained by linkage of the carboxy and amino termini of the protein.

10. A method according to any preceding claim, in which the prey protein is an antibody or a polypeptide selected from SH2, PTB, 14-3-3 and WW domain.

11. A method according to any preceding claim, in which the prey protein further comprises an epitope tag to enable rapid detection of fusion protein synthesis.

12. A tribrid cell engineered to express a cDNA library of enzymes or putative enzymes, and a prey protein for use in the method of claims 1 to 11.

13. A tribrid cell according to claim 12, in which the cell is a eukaryote cell.

14. A cell according to claim 12 or claim 13, in which the cell is a yeast cell

15. A method substantially as hereinbefore described with reference to and as illustrated by the Examples.

16. A cell substantially as hereinbefore described with reference to and as illustrated by the Examples.

CLAIMS

1. A screening method for regulatory enzymes, the method comprising the construction of tribrid cells containing genes encoding an expression library of putative enzymes, a bait protein or polypeptide fused to a known DNA binding domain and a prey protein which is an antibody that recognises a protein or polypeptide which has been post translationally modified, the prey protein being attached to a known protein active domain, whereby, in use, binding or recognition of the bait protein or polypeptide by the prey protein or polypeptide upon post-translational modification by an enzyme contained in the expression library, causes transcription of a reporter gene or genes which allow recognition of the enzyme activity.

2. A method according to claim 1, in which the cell is a eukaryote.

3. A method according to claim 1 or claim 2, in which the cell is a yeast cell.

4. A method according to any one of claims 1 to 3, in which the enzyme is involved in the post-translational modification of nascent proteins or polypeptides.

5. A method according to claim 4, in which the enzyme is involved in the regulation of phosphorylation, glycosylation, sulphonation, acetylation, side chain modification, nitrosylation, ubiquination, myristoylation or palmitoylation.

6. A method according to claim 4 or claim 5, in which the enzyme is a kinase or a phosphatase.

7. A method according to any preceding claim, in which the bait protein is an oncoprotein, a kinase, a phosphatase, a receptor protein, an adapter protein or a scaffolding protein.

8. A method according to any preceding claim, in which the prey protein is conformationally constrained within the cell.

9. A method according to claim 8, in which the prey protein is conformationally constrained by linkage of the carboxy and amino termini of the protein.

10. A method according to any preceding claim, in which the prey protein further comprises an epitope tag to enable rapid detection of fusion protein synthesis.

11. A tribrid cell for use in the method of claims 1 to 10, said tribrid cell being engineered to express an enzyme or putative enzyme from a cDNA library, a bait protein or polypeptide fused to a known DNA binding domain and a prey protein which is an antibody that recognizes a protein or polypeptide which has been post translationally modified, the prey protein being attached to a known protein active domain, whereby, in use, binding or recognition of the bait protein or polypeptide by the prey protein or polypeptide upon post-translational modification by the enzyme from the cDNA library causes transcription of a reporter gene or genes which allow recognition of the enzyme activity.

12. A tribrid cell according to claim 11, in which the cell is a eukaryote cell.

13. A cell according to claim 11 or claim 12, in which the cell is a yeast cell.

14. A method substantially as hereinbefore described with reference to and as illustrated by the Examples.

15. A cell substantially as hereinbefore described with reference to and as illustrated by the Examples.